DEVELOPMENT OF A CELL-BASED BIOSENSOR FOR COMPOUND DETECTION

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ABSTRACT

The threat of environmental pollution, biological warfare agent dissemination, and new diseases has in recent decades increased research into high throughput cell-based biosensors (Bousse, 1996; Gross et al., 1997; Jung et al., 1998). The creation of this class of biosensors could specifically aid in the detection of hazardous bioagents and other toxins. Understanding the validity and sensitivity of these sensors should also help with determining the mechanisms of drug- and chemical-induced toxicity (Davila et al, 1998). The current systems have been validated using a wide-range of toxins including synthetic pesticides, common heavy metals, and widely studied drugs used in treating cardiac dysfunction. We have tested these various bioagents on two different biosensor systems – MEAs and FETs, and have found that they can be used for testing in a high throughput platform for toxicity evaluation.

1. INTRODUCTION

The biosensors consist of a confluent monolayer of embryonic cardiac myocytes cultured on either microelectrode array (MEA) composed of sixty substrate-integrated electrodes or a field effect transistor array (FET) containing sixteen gates used as recording sites as indicated in Figure 1.

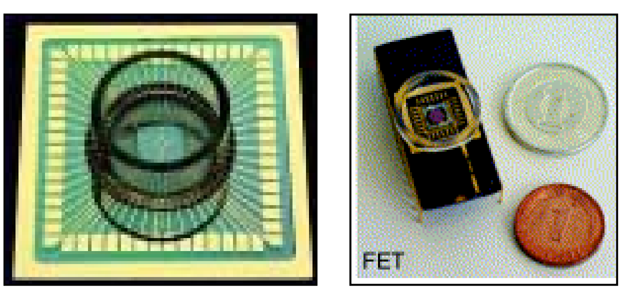

Figure 1: left) Substrate integrated MEA from Multichannel Systems and right) 4x4 array of positive channel FET (Offenhäusser et al., 1997)

In this study we developed a high-throughput methodology for detection of different chemical agents using MEA and FET extracellular recordings from cardiac myocytes. The cardiac myocytes were plated on the surface of the devices (Figure 2), cultured, and then tested using the various bioagents.

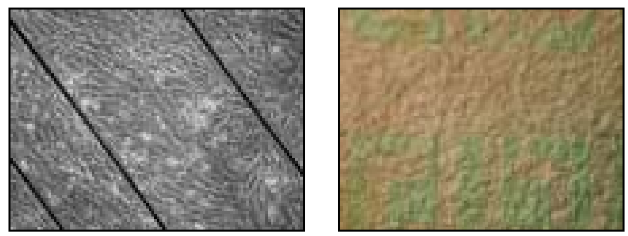

Figure 2: Cardiac cell growth on left) MEA surfaces and right) FET surfaces. Magnification: 20X

Spontaneous activity of the beating cells on MEAs produced extracellular field potentials in the range of 100 µV to nearly 1200 µV with a beating frequency of 0.5 to 4 Hz. The activity on the FETs produced average amplitudes of 1000 µV.

The specific compounds tested included environmental toxins such as pyrethroid pesticides, the heavy metals mercury and cadmium, epinephrine (Figure 3 and 4), and the antiarrhythmic drugs isoproterenol (ISO), verapamil (VP) and digoxin.

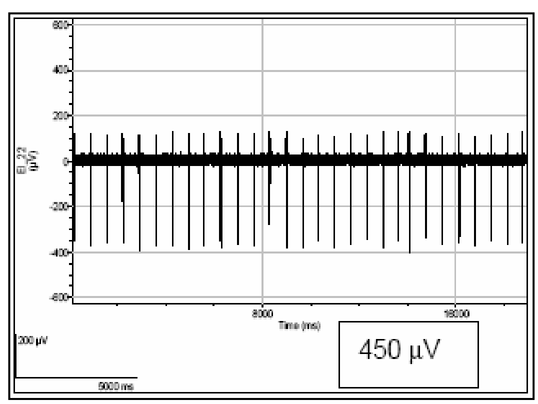

Figure 3: Field potential signals before addition of epinephrine, frequency: 1.33 Hz

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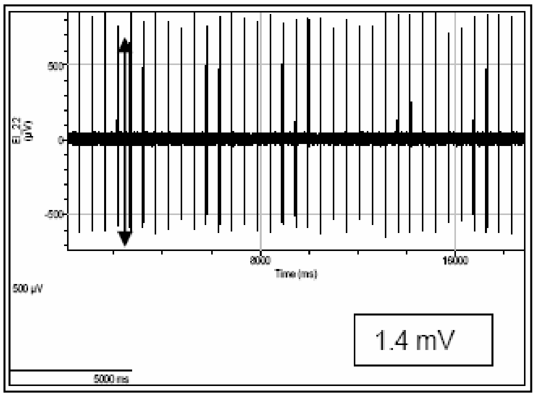

Figure 4: Field potential signals after addition of 10 µM epinephrine, frequency: 3.33 Hz

2. RESULTS

The compounds produced changes in the electrophysiological properties of the cardiac myocytes, namely inducing changes in the beating frequency and amplitude. Varied concentrations of each compound produced corresponding effects in the electrophysiological properties. The pyrethroid pesticides, α-cypermethrin, tetramethrin, and tefluthrin, reduced beating frequency and amplitude. The heavy metal cadmium (from $CdCl_2$) blocked calcium channel activity of the cardiac myocytes and caused a fast decrease in both the amplitude and frequency.

Decreasing concentrations of the antiarrhythmic agents also were used to test the biosensor's capabilities. VP and ISO were tested to determine MEA drug sensitivity. VP, a calcium channel blocker, and digoxin, a sodium ATPase inhibitor, showed decreased beating frequency in the cardiac monolayer. ISO, a beta adrenergic agonist, and epinephrine were shown to increase the beating rate. Each effect was specific to the particular compound tested.

3. CONCLUSION

The data produced from the cell-electrode and cell-transistor hybrid devices are being used to create a database of the effects of various toxins. This could then be used to identify unknown compounds exposed to the cell-based biosensor. Validation with toxins and drugs is the first step towards the creation of a high-throughput testing method for unknown toxicity evaluation.

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